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Review Article

Molecular targeted therapy with transarterial chemoembolization

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A B S T R A C T

Hepatocellular carcinoma (HCC) remains the third most common cause of cancer-related deaths worldwide, although improvements in patient stratification and the introduction of novel therapies have improved patient survival. Despite surveillance programs, 80% of HCCs are diagnosed at an advanced stage, at which point noncurative treatment, including transarterial chemoembolization (TACE) or sorafenib, is indicated. In intermediate stage HCC, suboptimal treatment outcomes are usually associated with a high rate of recurrence after TACE by eliciting a reaction from vascular endothelial growth factor. The modest anti-cancer benefits of sorafenib, an anti-angiogenic agent, coupled with its adverse effect profile are two additional barriers to overcome in treating advanced HCC. Considering the limitations of TACE and sorafenib in intermediate to advanced stage HCC patients, the potential benefits of combination therapy are attractive. Besides sorafenib, many novel agents are under investigation in Phase III trials of advanced HCC. However, to date nothing has been shown to perform better than sorafenib. Moreover, recently presented efficacy results evaluating a combination of TACE with molecularly targeted therapies including sorafenib are less impressive. While TACE or anti-angiogenic therapies results in tumor hypoxia and cell death, this may also activate hypoxia-induced survival signals including hexokinase II, carbonic anhydrase IX, or protein disulfide isomerase. To overcome this situation, the inhibition of hypoxia-induced survival signals might be additionally required as an adjuvant therapy following TACE or anti-angiogenic therapies. Therefore, further basic experiments and clinical studies are required to enhance the therapeutic potency of TACE for HCC.

Keywords: Hepatocellular carcinoma, Molecularly targeted therapy, Transarterial chemoembolization

Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths worldwide and the leading cause of death in patients with cirrhosis.[1,2] HCC is a complex disease that is dually challenging to treat due to underlying cirrhosis of the liver in addition to the cancer itself.[3] Despite surveillance programs conducted in high-risk populations, most HCCs are diagnosed at an advanced stage. As a result, only 10–20% of patients are candidates for curative treatment. The remaining 80% of cases are diagnosed at intermediate or advanced stages for which noncurative treatment modalities are recommended. Therapeutically, Barcelona Clinic Liver Cancer (BCLC) intermediate stage HCC patients are considered optimal candidates for transarterial chemoembolization (TACE).[4] TACE delivers a chemotherapeutic agent into the feeding vessels of a HCC and produces ischemic insult by blocking subsequent perfusion of these vessels with a plugging material.[5] Two pivotal randomized controlled trials and a subsequent meta-analysis confirmed that TACE improved survival in patients with HCC.[6–9] However, intermediate stage HCC includes a heterogeneous population of patients that can vary widely in terms of tumor burden, liver function, and disease etiology.[10] A high rate of recurrence and unsatisfactory treatment outcomes after TACE due to large tumor size and a high number of tumors remains troublesome.[11]

Sorafenib, an anti-angiogenic multi-kinase inhibitor, is the first systemic therapy to demonstrate improved survival in patients with advanced HCC [10.7 months vs. 7.9 months in the Sorafenib HCC Assessment Randomized Protocol (SHARP) trial; 6.5 months vs. 4.2 months in an Asia-Pacific trial].[12,13] Given this modest survival gain and the limitations of sorafenib therapy, such as resistance and tolerability, there are still unmet needs in the treatment of advanced stage HCC. Currently, multifocal (intermediate BCLC stage B) HCC is treated primarily with TACE alone and more advanced (BCLC stage C) HCC is treated with sorafenib monotherapy.[4] Considering that subsequent recurrence and death is common in intermediate to advanced stage HCC patients and these treatments produce a modest survival benefit (median survival time is about 11–20 months) when used alone,[14] the potential benefits of combination therapy are attractive.[10] Recently emerging molecularly targeted therapies appear to be promising agents in prolonging the overall survival of late stage HCC patients[15] and these therapies are considered good partners for TACE. However, anti-angiogenic therapies including TACE cause tumor hypoxia leading to an upregulation in hypoxia inducible factor-1α (HIF-1α), which in turn activates hypoxia-induced survival signals that may promote HCC progression.[16] Therefore, understanding and controlling these signals may be essential in maximizing the efficacy of TACE.

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This review discusses the mechanisms of combination therapy using molecularly targeted therapy with TACE and clinical studies that have investigated these therapies in patients with HCC. Perspectives for future developments to improve treatment outcomes in HCC are also provided.

Combination of sorafenib and TACE

Although TACE embolizes the major feeding arteries of a tumor, it leaves smaller vessels open, which explains why the procedure is considered palliative and not curative.[5] Moreover, in cases of multifocal HCC with a high burden of radiologically invisible foci, these additional lesions cannot be treated with TACE and TACE may even promote their growth. TACE induces central anoxia with peripheral hypoxia. This hypoxic stress provokes cells to release angiogenic growth factors including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF).[17–19] It is well documented that circulating levels of VEGF increase after TACE[20] and the use of anti-angiogenic therapy in combination with transarterial embolization (TAE) is supported in a preclinical model. This combination causes a reduction in tumor volume and vessel density, as well as a prolongation in survival compared with TAE alone.[21] Therefore, one improvement in the treatment of HCC with TACE would be to prevent the recruitment of these secondary vessels.

Sorafenib targets and markedly suppresses serine/threonine kinases of Raf in the MAP kinase cascade, stem cell factor receptor (c-kit), and inhibits the tyrosine kinases of angiogenesis factor receptors such as VEGF and PDGF receptors.[22] Sorafenib thus simultaneously prevents the proliferation of intrahepatic microscopic remnant tumors after TACE and inhibits angiogenesis.[23] In addition, the ability of sorafenib to induce apoptosis, possibly by inhibiting the MEK/ERK-independent effects of Raf-1, in a wide variety of human tumor cell lines, could complement the cytotoxic effects of standard chemotherapies by re-sensitizing resistant tumor cells.[24,25] Since TACE allows for a combined effect of targeted delivery of chemotherapeutic agents with ischemia induced by embolization,[11] sorafenib may augment the efficacy of TACE not only by inhibiting angiogenesis, but also by enhancing the therapeutic efficacy of delivered chemotherapeutic agents through the induction of apoptosis.

Based on these possible synergistic effects, there are a substantial number of clinical trials assessing the combination of TACE with sorafenib that have either been completed or are currently underway. The combination of TACE and anti-angiogenic therapy has to involve careful consideration of the timing of TACE in relation to the anti-angiogenic therapy.[26] That timing may be critical was shown in the TACE and sorafenib study published recently by Kudo et al,[27] wherein sequential sorafenib therapy demonstrated no added benefit. Patients with unresectable HCC were randomized, 229 each to a sorafenib or placebo group starting 1–3 months after 1–2 sessions of TACE. The median time-to-progression (TTP) in the sorafenib group was not significantly different from the placebo group [5.4 versus 3.7 months, respectively; hazard ratio, 0.87; 95% confidence interval (CI), 0.70–1.09; $P = 0.252$]. The different outcomes reported by Pawlik et al (a disease control rate of 95% and an objective response of 58%)[28] compared with those of Kudo et al,[27] may be due to the timing of sorafenib. In an attempt to explain the biologic effects of combining anti-angiogenic therapies and TACE, three models have been proposed: sequential, interrupted, and continuous.[29] The first two models address the risk of bleeding from continuing sorafenib at the time of an invasive vascular procedure like TACE. The third, a continuous administration approach, aims at inhibiting the surge of VEGF after TACE that rises to a peak on Day 1 and then falls, suggesting that sorafenib may exert its greatest anti-angiogenic effects when administered before or immediately after TACE.[30] The Phase II randomized, double-blind, placebo-controlled sorafenib or placebo in combination with TACE in HCC (SPACE) trial incorporated a continuous sorafenib administration protocol concurrently with Drug-eluting bead TACE (DEB-TACE). While the study met its primary endpoint of improving TTP when sorafenib was added to a regimen of DEB-TACE, the median TTP reported was similar for both groups (5.6 vs. 5.5 months, respectively).[10] The optimal clinical approach will depend on a balance between safety and efficacy. Positive data from current research efforts directed at combining molecularly targeted agents with TACE are needed to recommend this novel therapeutic approach.

Another important clinical concept is whether TACE, as a powerful complimentary armament to sorafenib, could be allowed for patients with advanced HCC. This approach needs to be validated and the results of an ongoing randomized, controlled Phase III trial (STAH study) of sorafenib with or without conventional TACE in patients with advanced HCC (NCT01829035) is eagerly awaited.

Combination of other molecularly targeted therapies and TACE

Due to genetic heterogeneity, some HCC cells are initially resistant to sorafenib; this is termed primary resistance.[3] Multiple molecular pathways implicated in the pathogenesis of HCC are now being evaluated as potential targets for therapeutic interventions, including VEGF, fibroblast growth factor (FGF), PDGFR, epidermal growth factor receptor (EGFR), and mammalian target of rapamycin (mTOR) pathways.[22] In addition, there are many other novel agents and targets in development, including histone deacetylase inhibitors (HDAC), c-Met inhibitors, MEK kinase inhibitors, insulin-like growth factor receptor (IGFR), arginine deiminase, and the anti-VEGF monoclonal antibody bevacizumab.[22] Among these novel agents, brivanib is a selective dual inhibitor of the FGF and VEGF receptors that is currently in being investigated in a Phase III trial (the BRISK-TA study) as adjuvant therapy following TACE in 870 patients with intermediate HCC and compared to placebo (NCT00908752).[22] TSU-68 (orantinib) is an oral, small molecule inhibitor of VEGFR, PDGFR, and FGFR also currently being evaluated in a Phase III study (ORIENTAL) in combination with TACE versus TACE alone (NCT01465464).[22] In a pilot study, patients who were scheduled to undergo TACE were randomized to either observation (TACE-O) or intravenous bevacizumab (TACE-BEV). The progression-free survival at 16 weeks was 0.19 in the TACE-O arm and 0.79 in the TACE-BEV arm ($P = 0.021$). However, the median OS was similar in both arms [61 months in the TACE-O arm and 49 months in the TACE-BEV arm ($P = 0.21$)].[31]

Future perspectives: Experimental agents inhibiting hypoxia-induced survival signals in HCC

Recently presented efficacy results from multicenter, randomized, placebo-controlled clinical trials evaluating a combination of molecularly targeted therapies, including sorafenib with TACE, are less impressive in terms of marginal survival benefit. While inhibition of angiogenesis results in tumor hypoxia and cell death, this may also activate hypoxia-induced cell survival signals, which might promote HCC progression. Therefore, we briefly summarize these hypoxia-induced survival signals as novel therapeutic targets and promising adjuvant therapies for TACE.

Hexokinase II

In hypoxic HCC cells, a glycolytic system induced by hypoxia-inducible factor-1α (HIF-1α) operates as a salvage pathway to

produce ATP.[32] Hexokinase II (HK-II) is a key enzyme in this system and might be over-expressed in HCC.[33] This enzyme phosphorylates glucose, trapping the substrate in the cytoplasm and facilitating further glycolysis. We found that hypoxia enhanced HCC cell growth through HIF-1α-dependent HK-II induction. The HK-II inhibitor (3-bromopyrubate, 3-BP) significantly inhibited cellular growth in hypoxic conditions compared to cells in normoxic conditions. This suppression was caused by the induction of HCC cell apoptosis via activating the mitochondrial apoptotic pathway in hypoxic cells.[34] In another *in vitro* study, we demonstrated that hypoxia enhanced mitochondrial stability, which was inhibited by 3-BP treatment.[35]

In addition, the effect of 3-BP on suppression of tumor growth was confirmed in an *in vivo* animal model of HCC. *In vivo*, the HCC mouse model was established by intradermal implantation of MH134 cells in C3H mice. The mean tumor volume and tumor volume growth were significantly reduced in mice treated with 3-BP. Moreover, the degree of apoptosis quantified by terminal deoxyducleotidyl transferase-mediated dUTP nick-end labeling staining and 99mTc-hydrazinonicotinamide annexin V imaging was significantly reduced after 3-BP treatment.[35] These *in vitro* and *in vivo* studies collectively show that hypoxia enhances mitochondrial stability through HK-II induction and that treatment with the HK-II inhibitor 3-BP enhances apoptosis via a mitochondrial pathway in HCC under hypoxic conditions.

Carbonic anhydrase IX

Glycolysis produces a lot of lactate and carbon dioxide (CO_2) as by-products. The resulting acidic microenvironment causes transient intracellular acidification, which is incompatible with cell growth and survival. Carbonic anhydrase-IX (CA-IX) is a hypoxia-inducible transmembrane protein and is involved in lowering pH by expediting the pericellular metabolism of CO_2 in collaboration with bicarbonate transporters.[36] There is a significant correlation between CA-IX expression and the area of hypoxia in solid tumors, suggesting that this enzyme plays an important role in the adaptation of tumor cells in hypoxia.[37] Several clinico-pathological studies indicate that high CA-IX expression in tumors is closely related to poor prognosis and CA-IX is one of the important survival mechanisms in cancer cells under acidic environments caused by hypoxia.[38]

Our *in vitro* study confirmed that HCC cells also expressed CA-IX and that its expression was increased in cells cultured under hypoxic conditions as compared with normoxic cells.[39] Even though CA-IX inhibition alone failed to induce significant HCC cell growth suppression or apoptosis, the inhibition of CA-IX enhanced 3-BP-induced apoptotic cell death mediated by endoplasmic reticulum (ER) stress-dependent Jun N-terminal kinase (JNK) activation.[39] In addition, HCC tissues express CA-IX and tumoral CA-IX intensity is inversely related with E-cadherin intensity, thus potentiating invasiveness and metastasis.[39] Therefore, blocking CA-IX in combination with an HK-II inhibitor might be a useful therapeutic strategy for hypoxic HCCs.

Protein disulfide isomerase

The ER fulfills multiple cellular functions. Because of its role in protein folding and transport, the ER is also rich in Ca^{2+}-dependent molecular chaperones, which stabilize protein-folding intermediates. Many disturbances, including glucose deprivation, misfolded proteins, oxidative stress, and hypoxia can trigger accumulation of unfolded proteins in the ER and lead to ER stress. The unfolded protein response (UPR) is an important ER stress response which saves the cell by removing unfolded or misfolded proteins.[40] In particular, activation of transcriptional induction of ER chaperones, such as protein disulfide isomerase (PDI), is involved in restoring protein folding activity.[41] However, excessive and prolonged ER stress elicits apoptotic cell death.

It was recently reported that 3-BP, an HK-II inhibitor, may induce ER stress and thereby cause apoptosis in human HCC cell lines.[39] When ER stress is induced by 3-BP, UPR might be activated as a compensatory mechanism. Thus, we postulated that blocking this activity may augment ER stress and enhance 3-BP-induced apoptosis. We adopted bacitracin as a PDI inhibitor to examine the functional profiles of PDI in HCC cells. The peptide antibiotic bacitracin was reported to be an inhibitor of PDI in 1981, and since then has been widely used to demonstrate the role of PDI in cellular processes.[42] Indeed, when bacitracin-treated HCC cells were treated with 3-BP under hypoxic conditions, apoptosis was significantly enhanced as compared to cells only treated with 3-BP. We then found that pro-apoptotic JNK was more promptly and potently activated in cells treated with 3-BP and PDI inhibitor than in cells only treated with 3-BP. In animal experiments, mean tumor volumes were significantly reduced in mice treated with 3-BP and bacitracin as compared with controls, 3-BP, or bacitracin-treated mice.[43] NONMEM analysis showed that 3-BP and bacitracin had a synergistic anti-tumor effect. Therefore, a combination of PDI inhibitor and HK-II inhibitor may be therapeutically beneficial for rapidly growing hypoxic HCCs.

Conclusion

The management of HCC has changed substantially in the past few decades based on recent advances in the understanding of HCC pathophysiology and the development of new therapies. Based on new molecular knowledge and recognition of the limitations of sorafenib, novel molecularly targeted therapies and combination strategies have been developed. Although early phase data with these agents have looked promising, to date nothing has been shown to be better than sorafenib. Incorporating these new agents as an adjuvant therapy for TACE provides an opportunity to increase our understanding of these agents in HCC. Moreover, inhibition of hypoxia-induced survival signals might be additionally required as adjuvant therapy following TACE or anti-angiogenic therapies commonly result in significant tumor hypoxia. Therefore, further basic experiments and clinical studies are required to enhance the therapeutic potency of TACE in the treatment of HCC.

Conflicts of interest

None.

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